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Effect of Cyclophosphamide on the Glycogen Level of Tumor Bearing Mice

Urmi SEN, MAITREYI Guha THAKURTA
and J. Roy CHOWDHURY

Department of Tumor Biology, Chittaranjan National Cancer Research Centre, Calcutta-700 026 INDIA

ABSTRACT

The dependance of transplanted tumors on mobilization of host's liver glycogen and the responsiveness of the latter towards tumor inhibition by therapeutic means were explored in Swiss mice. Sarcoma 180 and fibrosarcoma were grown in Swiss mice and treated with cyclophosphamide in the dosage of 25 mg/kg body weight for seven consecutive days; starting therapy I, 5 days after transplantation, as well as II, 8 days after transplantation. Mobilization of liver glycogen was observed as a function of tumor growth, which was evidenced as early as fifth day of transplantation by a sharp fall of glycogen level. While lowering of glycogen level have been reported in case of hepatoma and leukemic liver, the present result indicates fall of host's liver glycogen as a result of growth of the transplanted tumors of different origin. The responsiveness of the tumors to cyclophosphamide therapy by tumor inhibition is concomittant with elevation of liver glycogen. The tumor which escaped inhibitory effect of the drug did not show such expression of liver glycogen. The present result indicates that growth and control of growth of neoplastic cells have direct relationship with host's liver glycogen.

INTRODUCTION

Glycogen is the main carbohydrate reserve of animal tissue, which occurs in different forms of varying degrees of polymerization. It is stored primarily in the liver (1), although the distribution pattern is widespread in different tissues including cervical epithelium. Glycogen has been shown to be a sensitive indicator of cellular differentiation, with significant decrease of its content in neoplasia of cervical epithelium (2～6). In animal experiments it is observed that tumor tissue is associated with low glycogen content (7) ,and this phenomenon is strikingly focussed in the comparative study of normal and neoplastic liver (8).

There are scanty reports on the effects of transplanted tumor growth

on host liver glycogen. While rat liver bearing leukemic strains show depletion of liver glycogen from that of normal rats (9, 10), however liver of rats bearing transplantable hepatoma show variable results (11, 12). In the present study mobilization of liver glycogen has been demonstrated as a result of transplantation of Sarcoma 180 as well as fibrosarcoma in Swiss mice.

Since glycogen has been shown to be a sensitive indicator of different types of malignancies, attention has been focussed on its behavioural pattern during tumor regression under therapeutic measures. The present study furnishes information with respect to responsivenss of liver glycogen towards tumor inhibition by cyclophosphamide.

MATERIALS AND METHODS

Tumor Strain. Two different tumor strains have been used in this study.

1. Fibrosarcoma — This is maintained as a solid tumor by serial transplantation in Swiss mice and grows subcutaneously in the inguinal region with an inoculum size of 5×10^7 cells/0.5 ml.

2. Sarcoma 180 (S-180) — This is maintained in the ascitic form by serial transplantation of 3.7×10^7 cells/0.5 ml by i. p. injection. Mice so inoculated develop adequate amount of ascitic fluid in 3～5 days.

Swiss mice bearing each tumor strain were used in the experiment for therapy. An equal number of mice were used as controls. The experimental animals were divided into two groups. In group I treatment was started on 5th day after tumor transplantation. In group II treatment was starte on 8th day of tumor transplantation.

Administration of Cyclophosphamide. Cyclophosphamide which is marketed under the trade name of Endoxan (obtained from Khandelwal Laboratoris Pvt. Ltd., under agreement with Asta-Werk, A. G. Bracherede, West Germany) was used in the dosage of 50 mg/kg/day per mouse (13), and administered i. p. for 7 consecutive days. Control mice received equal volume of distilled water which is the solvent of Endoxan. Tumor volume in case of S-180 and tumor diameter in case of fibrosarcoma were noted. The livers and tumor tissues of the treated series were analysed for glycogen along with those of control series.

Glycoqen Localization. Glycogen was measured by the technique of Plummer (14). The tissues were digested with cold 5% T. C. A. repeatedly, and the filtrate was precipitated with ethanol. The precipitate was dissolved in water, and colour developed by heating with anthrone reagent. The bluish green colour was read at the spectrophotometer at 620 mμ. The glycogen per 100 g of tissue was calculated from the following formula :

$$\text{Glycogen} = \frac{Du}{Ds} \times 0.1 \times \frac{\text{volume of extract}}{\text{gm. of tissue}} \times 100 \times 0.9$$

Du = It represents optical density of the unknown
Ds = It represents optical density of standard

In case of fibrosarcoma the rate of tumor regression was noted by measuring the tumor diameter with slide calipers in both treated and control series.

In case of S-180 the tumor regression was measured by noting the fluid volume after sacrificing the animals. Cell counting was also done of the ascitic fluid and obtained as number of cells/ml of fluid.

RESULTS

The mobilization of liver glycogen expressed as mg/g of tissue in mice bearing both the tumor strains is shown in Fig. 1. It is evident that glycogen level decreases appreciably from 3rd day of transplantation onwards up to 7th day. Thereafter there is continuous but gradual slope indicating further decrease of liver glycogen although the rate of decrease become slow. Both the tumor strains demonstrate similar phenomenon of glycogen mobilization observed up to 21st day of transplantation.

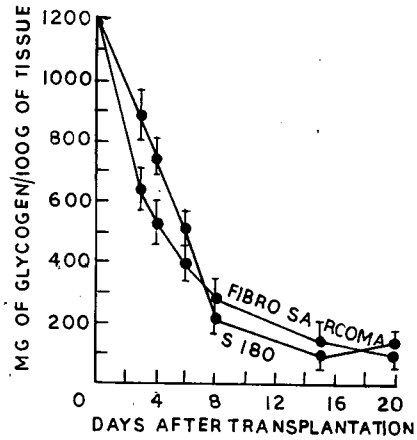

Fig. 1. Fall of liver glycogen as a result of transplantation of fibrosarcoma and sarcoma 180 in mice. Each point in the curve is an average of 15 to 20 animals. Scatter is within permissible limits.

The growth pattern of S-180 measured in terms of fluid volume, shows that after a short latent period, it enters the log phase of growth, followed by the lag phases at a later period (Fig. 2). It may be noted that the tumor take with respect to S-180 is almost 100%. In the treated group where the therapy was started on 5th day after tumor transplantation the regression pattern reveals that the fluid volume of S-180 does not increase and remains inhibited till 8th day after which there is a tendency for slow and gradual increase. When the treatment was started on the 8th day of transplantation, there is an almost linear negative slope of tumor growth up to 15th day of transplantation, after which there is a tendency for slow and gradual increase.

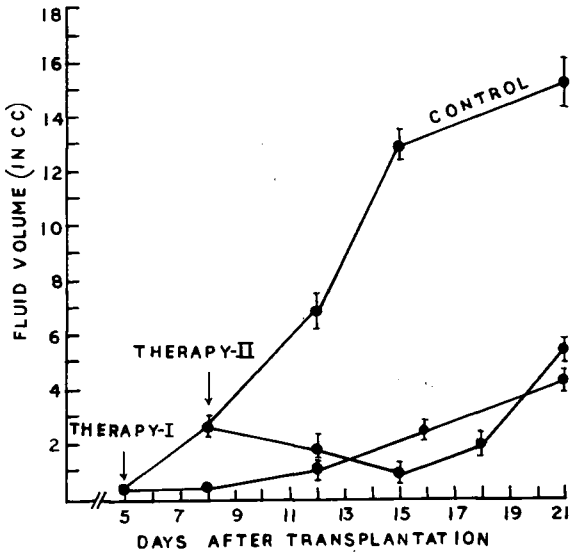

Fig. 2. Growth pattern of S-180 measured in term of fluid volume in control and treated series. Each point in the control curve is an average of 15–20 animals. Treated I is where the treatment was started 5 days after transplantation and treated II is where the treatment was started 8 days after transplantation. Each point in the treated curves is an average of 10 to 15 animals, which have shown tumor inhibition as a result of therapy. Mice which did not respond to therapy followed the same pattern as that of control curves. Significance test shows P values less than 0.2 for difference between control and treated groups during the entire period under study. However there is variation of P values.

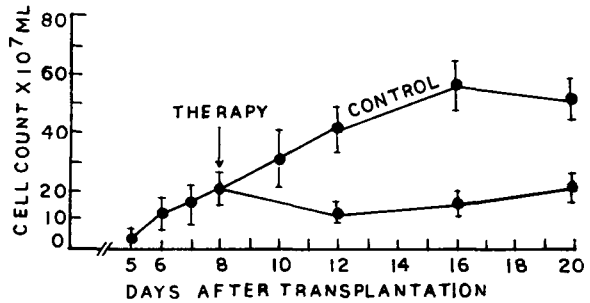

Fig. 3. Cell count of S-180 in control and treated series with time. Each point in the control curve is an average of 10–15 animals. Each point in the treated curve is an average of 10–15 animals, who have responded to therapy. Significance test shows P value less than 0.2 for difference between control and treated groups during the entire period under study. However there is variation of P values.

Cell counting was done in both the control group and the treated group, where treatment was started on 8th day of transplantation. The result revealed that along with decrease in the tumor volume with respect to therapeutic stress, there is simultaneous decrease in the number of viable tumor cells per ml of fluid (Fig. 3). In both the Figures 2 and 3 the curves denote values in S-180 bearing mice, which have responded to cyclophosphamide therapy which is about 60～65% in our series in case of group I and less in group II. The tumor which escaped cyclophosphamide action and did not show inhibition followed the same pattern of growth as that of control.

The regression pattern of fibrosarcoma is expressed in terms of mean diameter of the tumors (Table 1). The response of fibrosarcoma to therapy in this laboratory is 50～52%. The values given in Table 1 under the treated series denotes those which have responded to therapy. The tumor is under check upto 16th day of transplantation after which there is a tendency to increase in size. However the measurement given as mean diameter is not the absolute indicator of the size, as the treated tumors are often seen to be accompanied by a significant amount of necrotic material, which should not come into consideration.

The comparative values of liver glycogen in both treated and untreated series of the two tumor strains have been depicted in Fig. 4. The evaluation was made on the 15th day of transplantation in case of group I and 21st day of transplantation in case of group II, taking control untreated mice of the corresponding day.

In group I where the treatment was started 5 days after transplantation

Table 1. *Mean Diameter of the Fibrosarcoma with Respect to Cyclophosphamide Therapy*

Days after fibrosarcoma transplantation	Control Group	Treated Group
8th	1.4	1.4
12th	2.0	1.7
16th	2.8	1.75
18th	3.0	2.1
21st	3.5	2.6

Size of tumor (fibrosarcoma)[a] at different times after transplantation both in the treated and control series. The treatment was started on the 8th day of transplantation. Values shown are means and each is an average of 15-20 animals.

a Measurements are given in cm.

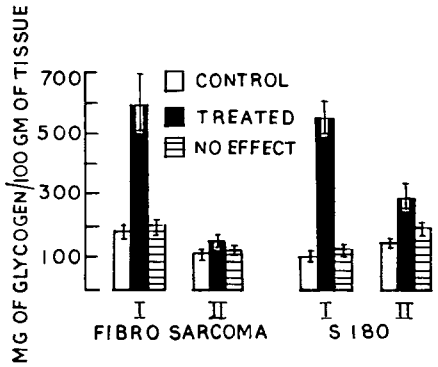

Fig. 4. Comparative values of liver glycogen in treated and untreated series of mice bearing fibrosarcoma and S-180. I denotes values where the treatment was started 5 days after the transplantation and evaluation done on 15th day of transplantation. II denotes values where the treatment was started 8 days after transplantation and evaluation done on 21st day of transplantation. The no effect bar denotes of liver glycogen in the treated series where the tumor did not respond to therapy. All values are average of 30 to 40 animals. Significance test shows P value less than 0.5 in all cases.

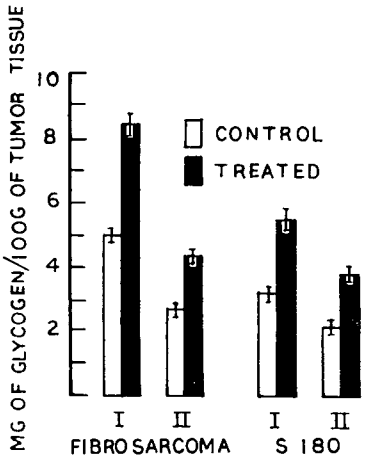

Fig. 5. Comparative values of the fibrosarcoma and S-180 tumor glycogen in control and treated animals. I denotes the values where the treatment was started 5 days after transplantation and evaluation was done on 15th day of transplantation. II denotes values where the treatment was started 8 days after transplantation and evaluation was done on 21st day of transplantation. The treated bar denotes values where the tumors have responded to therapy. All values are average of 20–25 mice. Significance test shows P values less than .05 in all cases.

the control liver glycogen shows greater value than that of group II, where the treatment was started after 8 days of transplantation and the control liver glycogen shows very low level of glycogen in both the tumor bearing mice. Strangely enough in group I there is a significant rise of glycogen level in liver as a result of cyclophosphamide therapy. Mice which did not show therapeutic responses of tumor inhibition, show no appreciable rise of liver glycogen as denoted in the no effect bar of the figure. In case of group II however, compared to the very low value of liver glycogen in the control, the elevation due to therapy was of much lesser magnitude.

In case of glycogen content of tumor tissues both in treated and untreated series (Fig. 5) it is noticed that, although the overall glycogen is at a very low level, there is significant elevation of glycogen in the treated series of both the groups which have responded to therapy.

DISCUSSION

It has been shown by many workers that experimental hepatoma contains low levels of glycogen as compared to that of normal liver (15, 16, 17). However there has been some differential reports regarding lowering of glycogen level in host liver of animals bearing transplanted hepatoma. While some transplanted hepatomas in rats were not shown to have caused depletion of liver glycogen, however, it was felt that other varieties of hepatoma particularly Novikoff hepatoma caused considerable lowering of liver glycogen in host rats (11, 18). Similar reports are obtained with respect to transplanted rat leukemia (9, 10). With respect to other transplanted tumors, the scanty reports that have been available deal with rapid utilization of host circulating glucose during growth of Ehrlich ascites cells and a few reports of loss of glycogen is reflected in the liver of tumor bearing animals (19). The present investigation establishes the significant mobilization of liver glycogen in mice as a result of growth of different transplanted tumors both in solid and ascitic form. As glycogen participates in the energy requirement of the cell (10, 20) its disappearance from the liver with the development of tumor can be well correlated with high growth potentials of the tumor cells along with greater metabolic competence of the cells.

Cyclophosphamide is a potent anticancer drug with significant effect of tumor inhibition in a variety of tumosr strains (13). The check of neoplastic growth due to the effectiveness of this chemotherapeutic drug as demonstrated in the present result has caused significant revival of liver glycogen.

The failure of elevation of liver glycogen in the treated series where therapeutic responses have not occured strongly suggests that revival of liver

glycogen is rather a function of control of neoplastic process than direct drug action.

The arrest of proliferative potency of tumor cells under the therapeutic stress and revival of liver glycogen under such condition assume great significance in the light of glycogen as an indicator of control of neoplastic process.

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